TGFb+MMP+ifn b Sequence

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MetProProS erGlyLeuAr gLeuLeuPro LeuLeuLeuP roLeuLeuTr pLeuLeuVal

CTGACGCCTG GCCCGCCGGC CGCGGGACTA TCCACCTGCA AGACTATCGA CATGGAGCTG    120
LeuThrProG lyProProAl aAlaGlyLeu SerThrCysL ysThrIleAs pMetGluLeu

GTGAAGCGGA AGCGCATCGA GGCCATCCGC GGCCAGATCC TGTCCAAGCT GCGGCTCGCC    180
ValLysArgL ysArgIleGl uAlaIleArg GlyGlnIleL euSerLysLe uArgLeuAla

AGCCCCCCGA GCCAGGGGGA GGTGCCGCCC GGCCCGCTGC CCGAGGCCGT GCTCGCCCTG    240
SerProProS erGlnGlyGl uValProPro GlyProLeuP roGluAlaVa lLeuAlaLeu

TACAACAGCA CCCGCGACCG GGTGGCCGGG GAGAGTGCAG AACCGGAGCC CGAGCCTGAG    300
TyrAsnSerT hrArgAspAr gValAlaGly GluSerAlaG luProGluPr oGluProGlu

GCCGACTACT ACGCCAAGGA GGTCACCCGC GTGCTAATGG TGGAAACCCA CAACGAAATC    360
AlaAspTyrT yrAlaLysGl uValThrArg ValLeuMetV alGluThrHi sAsnGluIle

TATGACAAGT TCAAGCAGAG TACACACAGC ATATATATGT TCTTCAACAC ATCAGAGCTC    420
TyrAspLysP heLysGlnSe rThrHisSer IleTyrMetP hePheAsnTh rSerGluLeu

CGAGAAGCGG TACCTGAACC CGTGTTGCTC TCCCGGGCAG AGCTGCGTCT GCTGAGGAGG    480
ArgGluAlaV alProGluPr oValLeuLeu SerArgAlaG luLeuArgLe uLeuArgArg

CTCAAGTTAA AAGTGGAGCA GCACGTGGAG CTGTACCAGA AATACAGCAA CAATTCCTGG    540
LeuLysLeuL ysValGluGl nHisValGlu LeuTyrGlnL ysTyrSerAs nAsnSerTrp

CGATACCTCA GCAACCGGCT GCTGGCACCC AGCGACTCGC CAGAGTGGTT ATCTTTTGAT    600
ArgTyrLeuS erAsnArgLe uLeuAlaPro SerAspSerP roGluTrpLe uSerPheAsp

GTCACCGGAG TTGTGCGGCA GTGGTTGAGC CGTGGAGGGG AAATTGAGGG CTTTCGCCTT    660
ValThrGlyV alValArgGl nTrpLeuSer ArgGlyGlyG luIleGluGl yPheArgLeu

AGCGCCCACT GCTCCTGTGA CAGCAGGGAT AACACACTGC AAGTGGACAT CAACGGGTTC    720
SerAlaHisC ysSerCysAs pSerArgAsp AsnThrLeuG lnValAspIl eAsnGlyPhe

ACTACCGGCC GCCGAGGTGA CCTGGCCACC ATTCATGGCA TGAACCGGCC TTTCCTGCTT    780
ThrThrGlyA rgArgGlyAs pLeuAlaThr IleHisGlyM etAsnArgPr oPheLeuLeu

CTCATGGCCA CCCCGCTGGA GAGGGCCCAG CATCTGCAAA GCGAATTCGG GGGAGGCGGA    840
LeuMetAlaT hrProLeuGl uArgAlaGln HisLeuGlnS erGluPheGl yGlyGlyGly

TCCCCGCTCG GGCTTTGGGC GGGAGGGGGC TCAGCGGCCG CAATCAACTA TAAGCAGCTC    900
SerProLeuG lyLeuTrpAl aGlyGlyGly SerAlaAlaA laIleAsnTy rLysGlnLeu

CAGCTCCAAG AAAGGACGAA CATTCGGAAA TGTCAGGAGC TCCTGGAGCA GCTGAATGGA    960
GlnLeuGlnG luArgThrAs nIleArgLys CysGlnGluL euLeuGluGl nLeuAsnGly
```

Fig. 1

TGFb+MMP+ifn b Sequence

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        10         20         30         40         50         60
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LysIleAsnL euThrTyrAr gAlaAspPhe LysIleProM etGluMetTh rGluLysMet

CAGAAGAGTT ACACTGCCTT TGCCATCCAA GAGATGCTCC AGAATGTCTT TCTTGTCTTC   1080
GlnLysSerT yrThrAlaPh eAlaIleGln GluMetLeuG lnAsnValPh eLeuValPhe

AGAAACAATT TCTCCAGCAC TGGGTGGAAT GAGACTATTG TTGTACGTCT CCTGGATGAA   1140
ArgAsnAsnP heSerSerTh rGlyTrpAsn GluThrIleV alValArgLe uLeuAspGlu

CTCCACCAGC AGACAGTGTT TCTGAAGACA GTACTAGAGG AAAAGCAAGA GGAAAGATTG   1200
LeuHisGlnG lnThrValPh eLeuLysThr ValLeuGluG luLysGlnGl uGluArgLeu

ACGTGGGAGA TGTCCTCAAC TGCTCTCCAC TTGAAGAGCT ATTACTGGAG GGTGCAAAGG   1260
ThrTrpGluM etSerSerTh rAlaLeuHis LeuLysSerT yrTyrTrpAr gValGlnArg

TACCTTAAAC TCATGAAGTA CAACAGCTAC GCCTGGATGG TGGTCCGAGC AGAGATCTTC   1320
TyrLeuLysL euMetLysTy rAsnSerTyr AlaTrpMetV alValArgAl aGluIlePhe

AGGAACTTTC TCATCATTCG AAGACTTACC AGAAACTTCC AAAACTGATC TAGACC       1376
ArgAsnPheL euIleIleAr gArgLeuThr ArgAsnPheG lnAsn...Se rArg
                                                 uga
```

ifn+MMP+TGFb Sequence

```
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MetAsnAsnA rgTrpIleLe uHisAlaAla PheLeuLeuC ysPheSerTh rThrAlaLeu

TCCATCAACT ATAAGCAGCT CCAGCTCCAA GAAAGGACGA ACATTCGGAA ATGTCAGGAG   120
SerIleAsnT yrLysGlnLe uGlnLeuGln GluArgThrA snIleArgLy sCysGlnGlu

CTCCTGGAGC AGCTGAATGG AAAGATCAAC CTCACCTACA GGGCGGACTT CAAGATCCCT   180
LeuLeuGluG lnLeuAsnGl yLysIleAsn LeuThrTyrA rgAlaAspPh eLysIlePro

ATGGAGATGA CGGAGAAGAT GCAGAAGAGT TACACTGCCT TTGCCATCCA AGAGATGCTC   240
MetGluMetT hrGluLysMe tGlnLysSer TyrThrAlaP heAlaIleGl nGluMetLeu

CAGAATGTCT TTCTTGTCTT CAGAAACAAT TTCTCCAGCA CTGGGTGGAA TGAGACTATT   300
GlnAsnValP heLeuValPh eArgAsnAsn PheSerSerT hrGlyTrpAs nGluThrIle

GTTGTACGTC TCCTGGATGA ACTCCACCAG CAGACAGTGT TTCTGAAGAC AGTACTAGAG   360
ValValArgL euLeuAspGl uLeuHisGln GlnThrValP heLeuLysTh rValLeuGlu

GAAAAGCAAG AGGAAAGATT GACGTGGGAG ATGTCCTCAA CTGCTCTCCA CTTGAAGAGC   420
GluLysGlnG luGluArgLe uThrTrpGlu MetSerSerT hrAlaLeuHi sLeuLysSer

TATTACTGGA GGGTGCAAAG GTACCTTAAA CTCATGAAGT ACAACAGCTA CGCCTGGATG   480
TyrTyrTrpA rgValGlnAr gTyrLeuLys LeuMetLysT yrAsnSerTy rAlaTrpMet

GTGGTCCGAG CAGAGATCTT CAGGAACTTT CTCATCATTC GAAGACTTAC CAGAAACTTC   540
ValValArgA laGluIlePh eArgAsnPhe LeuIleIleA rgArgLeuTh rArgAsnPhe

CAAAACGAAT TCGGGGGAGG CGGATCCCCG CTCGGGCTTT GGGCGGGAGG GGGCTCAGCG   600
GlnAsnGluP heGlyGlyGl yGlySerPro LeuGlyLeuT rpAlaGlyGl yGlySerAla

GCCGCACTAT CCACCTGCAA GACTATCGAC ATGGAGCTGG TGAAGCGGAA GCGCATCGAG   660
AlaAlaLeuS erThrCysLy sThrIleAsp MetGluLeuV alLysArgLy sArgIleGlu

GCCATCCGCG GCCAGATCCT GTCCAAGCTG CGGCTCGCCA GCCCCCCGAG CCAGGGGGAG   720
AlaIleArgG lyGlnIleLe uSerLysLeu ArgLeuAlaS erProProSe rGlnGlyGlu

GTGCCGCCCG GCCCGCTGCC CGAGGCCGTG CTCGCCCTGT ACAACAGCAC CCGCGACCGG   780
ValProProG lyProLeuPr oGluAlaVal LeuAlaLeuT yrAsnSerTh rArgAspArg

GTGGCCGGGG AGAGTGCAGA ACCGGAGCCC GAGCCTGAGG CCGACTACTA CGCCAAGGAG   840
ValAlaGlyG luSerAlaGl uProGluPro GluProGluA laAspTyrTy rAlaLysGlu

GTCACCCGCG TGCTAATGGT GGAAACCCAC AACGAAATCT ATGACAAGTT CAAGCAGAGT   900
ValThrArgV alLeuMetVa lGluThrHis AsnGluIleT yrAspLysPh eLysGlnSer

ACACACAGCA TATATATGTT CTTCAACACA TCAGAGCTCC GAGAAGCGGT ACCTGAACCC   960
ThrHisSerI leTyrMetPh ePheAsnThr SerGluLeuA rgGluAlaVa lProGluPro
```

Fig. 2

Ifn+MMP+TGFb Sequence

```
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 ValLeuLeuS erArgAlaGl uLeuArgLeu LeuArgArgL euLysLeuLy sValGluGln

 CACGTGGAGC TGTACCAGAA ATACAGCAAC AATTCCTGGC GATACCTCAG CAACCGGCTG   1080
 HisValGluL euTyrGlnLy sTyrSerAsn AsnSerTrpA rgTyrLeuSe rAsnArgLeu

 CTGGCACCCA GCGACTCGCC AGAGTGGTTA TCTTTTGATG TCACCGGAGT TGTGCGGCAG   1140
 LeuAlaProS erAspSerPr oGluTrpLeu SerPheAspV alThrGlyVa lValArgGln

 TGGTTGAGCC GTGGAGGGGA AATTGAGGGC TTTCGCCTTA GCGCCCACTG CTCCTGTGAC   1200
 TrpLeuSerA rgGlyGlyGl uIleGluGly PheArgLeuS erAlaHisCy sSerCysAsp

 AGCAGGGATA ACACACTGCA AGTGGACATC AACGGGTTCA CTACCGGCCG CCGAGGTGAC   1260
 SerArgAspA snThrLeuGl nValAspIle AsnGlyPheT hrThrGlyAr gArgGlyAsp

 CTGGCCACCA TTCATGGCAT GAACCGGCCT TTCCTGCTTC TCATGGCCAC CCCGCTGGAG   1320
 LeuAlaThrI leHisGlyMe tAsnArgPro PheLeuLeuL euMetAlaTh rProLeuGlu

 AGGGCCCAGC ATCTGCAAAG CtgaTCTAGA CC                                 1352
 ArgAlaGlnH isLeuGlnSe r...SerArg
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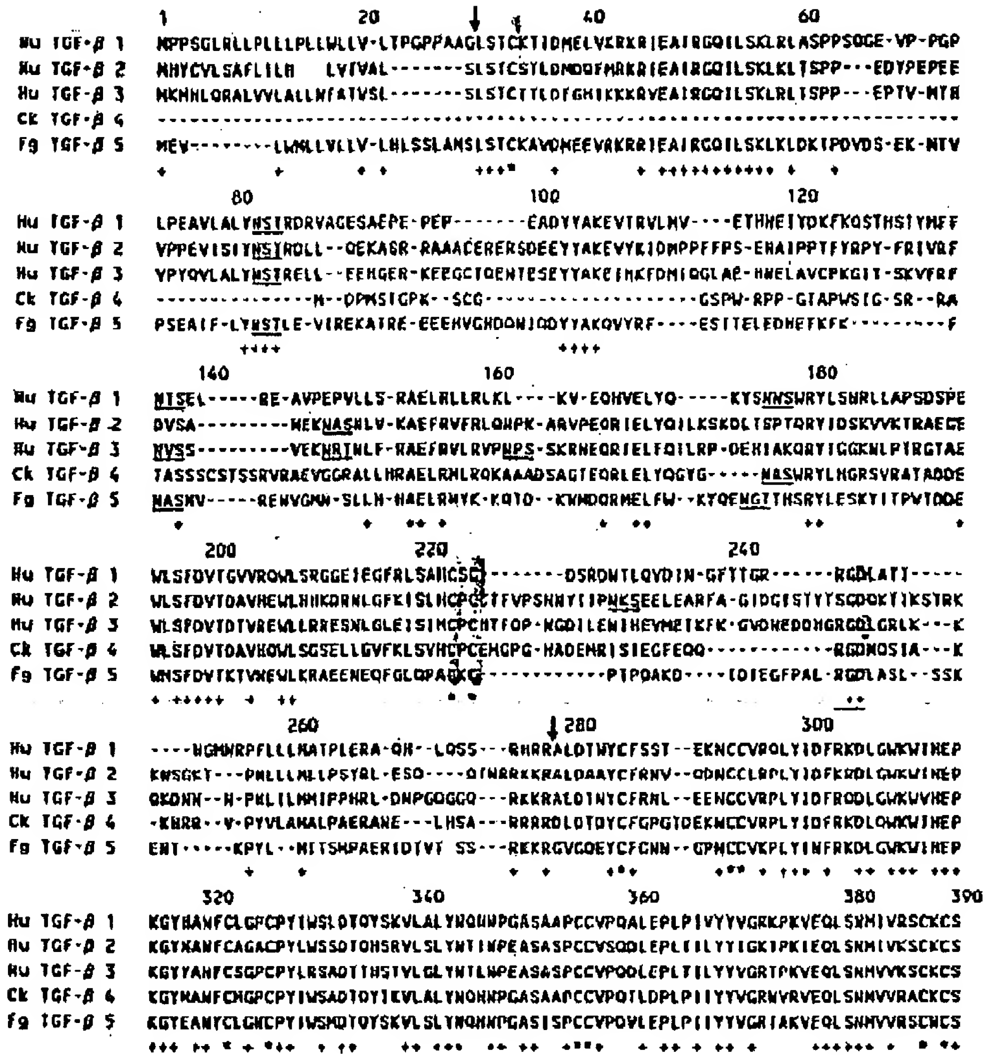


Fig. 3

Protein	Sequence	Reference
MMP-1/MMP-8		
Human type I collagen (α1)	Ala-Pro-Gln-Gly$_{775}$ ~ Ile$_{776}$-Ala-Gly-Gln	80
Human type I collagen (α2)	Gly-Pro-Gln-Gly$_{775}$ ~ Leu$_{776}$-Leu-Gly-Ala	80
Human type II collagen	Gly-Pro-Gln-Gly$_{775}$ ~ Leu$_{776}$-Ala-Gly-Gln	80
Human type III collagen	Gly-Pro-Leu-Gly$_{775}$ ~ Ile$_{776}$-Ala-Gly-Ile	80
Human α$_2$-macroglobulin	Gly-Pro-Glu-Gly$_{679}$ ~ Leu$_{680}$-Arg-Val-Gly	84
Rat α$_2$-macroglobulin	Ala-Ala-Tyr-His$_{681}$ ~ Leu$_{682}$-Val-Ser-Gln	84
Rat α$_2$-macroglobulin	Met-Asp-Ala-Phe$_{691}$ ~ Leu$_{692}$-Glu-Ser-Ser	84
Rat α$_1$-macroglobulin	Glu-Pro-Gln-Ala$_{683}$ ~ Leu$_{684}$-Ala-Met-Ser	84
Rat α$_1$-macroglobulin	Gln-Ala-Leu-Ala$_{685}$ ~ Met$_{686}$-Ser-Ala-Ile	84
Chicken ovostatin	Pro-Ser-Tyr-Phe$_{673}$ ~ Leu$_{674}$-Asn-Ala-Gly	79
Human pregnancy zone protein	Tyr-Glu-Ala–Gly$_{685}$ ~ Leu$_{686}$-Gly-Val-Val	84
Human pregnancy zone protein	Ala-Gly-Leu-Gly$_{687}$ ~ Val$_{688}$-Val-Glu-Arg	84
Human pregnancy zone protein	Ala-Gly-Leu-Gly$_{757}$ ~ Ile$_{758}$-Ser-Ser-Thr	84
α$_1$-Protease inhibitor	Gly-Ala-Met-Phe$_{352}$ ~ Leu$_{353}$-Glu-Ala-Ile	85
Human aggrecan	Ile-Pro-Glu-Asn$_{341}$ ~ Phe$_{342}$-Phe-Gly-Val	86
Human aggrecan	Thr-Glu-Gly-Glu$_{373}$ ~ Ala$_{374}$-Arg-Gly-Ser	86
Human cartilage link	Arg-Ala-Ile-His$_{16}$ ~ Ile$_{17}$-Gln-Ala-Glu	87
Human insulin-like growth factor binding protein-3	Leu-Arg-Ala-Tyr$_{99}$ ~ Leu$_{100}$-Leu-Pro-Ala	88
MMP-2		
Guinea pig α1(I) gelatin	Gly-Ala-Hyp-Gly$_{547}$ ~ Leu$_{548}$-Glx-Gly-His	24
Rat α1(I) gelatin	Gly-Pro-Gln-Gly$_{190}$ ~ Val$_{191}$-Arg-Gly-Glu	30
Rat α1(I) gelatin	Gly-Pro-Ala-Gly$_{277}$ ~ Val$_{278}$-Gln-Gly-Pro	30
Rat α1(I) gelatin	Gly-Pro-Ser-Gly$_{301}$ ~ Leu$_{302}$-Hyp-Gly-Pro	30
Rat α1(I) gelatin	Gly-Pro-Ala-Gly$_{331}$ ~ Glu$_{332}$-Arg-Gly-Ser	30
Rat α1(I) gelatin	Gly-Ala-Lys-Gly$_{361}$ ~ Leu$_{362}$-Thr-Gly-Ser	30
Rat α1(I) gelatin	Gly-Pro-Ala-Gly$_{382}$ ~ Gln$_{383}$-Asp-Gly-Pro	30
Rat α1(I) gelatin	Gly-Pro-Ala-Gly$_{634}$ ~ Phe$_{635}$-Ala-Gly-Pro	30
Rat α1(I) gelatin	Gly-Pro-Ile-Gly$_{676}$ ~ Asn$_{677}$-Val-Gly-Ala	30
Rat α1(I) gelatin	Gly-Pro-Hyl-Gly$_{685}$ ~ Ser$_{686}$-Arg-Gly-Ala	30
Bovine type I collagen (α1)	Gly-Pro-Gln-Gly$_{775}$ ~ Ile$_{776}$-Ala-Gly-Gln	22
Bovine type I collagen (α2)	Gly-Pro-Gln-Gly$_{775}$ ~ Leu$_{776}$-Leu-Gly-Ala	22
Human aggrecan	Ile-Pro-Glu-Asn$_{341}$ ~ Phe$_{342}$-Phe-Gly-Val	89
Human galectin-3	Pro-Pro-Gly-Ala$_{62}$ ~ Tyr$_{63}$-His-Gly-Ala	90
Human cartilage link	Arg-Ala-Ile-His$_{16}$ ~ Ile$_{17}$-Gln-Ala-Glu	87
Human cartilage link	Gly-Pro-His-Leu$_{25}$ ~ Leu$_{26}$-Val-Glu-Ala	87
Human insulin-like growth factor binding protein-3	Leu-Arg-Ala-Tyr$_{99}$ ~ Leu$_{100}$-Leu-Pro-Ala	88
MMP-3		
Human α$_2$-macroglobulin	Gly-Pro-Glu-Gly$_{679}$ ~ Leu$_{680}$-Arg-Val-Gly	79
Human α$_2$-macroglobulin	Arg-Val-Gly-Phe$_{684}$ ~ Tyr$_{685}$-Glu-Ser-Asp	79
Human α$_1$-antichymotrypsin	Leu-Leu-Ser-Ala$_{360}$ ~ Leu$_{361}$-Val-Glu-Thr	91
α$_1$-protease inhibitor	Glu-Ala-Ile-Pro$_{357}$ ~ Met$_{358}$-Ser-Ile-Pro	91
Antithrombin III	Ile-Ala-Gly-Arg$_{385}$ ~ Ser$_{386}$-Leu-Asn-Pro	91
Chicken ovostatin	Leu-Asn-Ala-Gly$_{677}$ ~ Phe$_{678}$-Thr-Ala-Ser	79, 92
Human aggrecan	Ile-Pro-Glu-Asn$_{341}$ ~ Phe$_{342}$-Phe-Gly-Val	93
Substance P	Lys-Pro-Gln-Gln$_{6}$ ~ Phe$_{7}$-Phe-Gly-Leu	37
Human ProMMP-1	Asp-Val-Ala-Gln$_{80}$ ~ Phe$_{81}$-Val-Leu-Thr	43
Human ProMMP-3	Asp-Thr-Leu-Glu$_{68}$ ~ Val$_{69}$-Met-Arg-Lys	94
Human ProMMP-3	Asp-Val-Gly-His$_{82}$ ~ Phe$_{83}$-Arg-Thr-Phe	94
Human ProMMP-8	Asp-Ser-Gly-Gly$_{78}$ ~ Phe$_{79}$-Met-Leu-Thr	95
Human ProMMP-9	Arg-Val-Ala-Glu$_{40}$ ~ Met$_{41}$-Arg-Gly-Glu	48
Human ProMMP-9	Asp-Leu-Gly-Arg$_{87}$ ~ Phe$_{88}$-Gln-Thr-Phe	48
Human fibronectin	Pro-Phe-Ser-Pro$_{689}$ ~ Leu$_{690}$-Val-Ala-Thr	21

Fig. 4

	Sequence	Reference
Human insulin-like growth factor binding protein-3	Leu-Arg-Ala-Tyr_{99}~Leu_{100}-Leu-Pro-Ala	88
	Ala-Pro-Gly-Asn_{109}~Ala_{110}-Ser-Glu-Ser	88
	Phe-Ser-Ser-Glu_{176}~Ser_{177}-Lys-Arg-Glu	88
Bovine α1(II) collagen, N-telopeptide	Ala-Gly-Gly-Ala_{113}~Gln_{114}-Met-Gly-Val	96
Bovine α1(II) collagen, N-telopeptide	Gln-Met-Gly-Val_{119}~Met_{120}-Gln-Gly-Pro	96
Bovine α1(IX) collagen, NC2	Met-Ala-Ala-Ser~Leu-Lys-Arg-Pro	96
Bovine α2(IX) collagen, NC2	~Ala-Lys-Arg-Glu	96
Bovine α3(IX) collagen, NC2	~Leu-Arg-Lys-Pro	96
Bovine α1(XI) collagen, N-telopeptide	Gln-Ala-Gln-Ala~Ile-Leu-Gln-Gln	96
Human cartilage link	Arg-Ala-Ile-His_{16}~Ile_{17}-Gln-Ala-Glu	87
Bovine insulin, B chain	Leu-Val-Glu-Ala_{14}~Leu_{15}-Tyr-Leu-Val	97
Bovine insulin, B chain	Glu-Ala-Leu-Tyr_{16}~Leu_{17}-Val-Cys-Gly	21, 97
MMP-7		
Human aggrecan	Ile-Pro-Glu-Asn_{341}~Phe_{342}-Phe-Gly-Val	89
Human cartilage link	Gly-Pro-His-Leu_{25}~Leu_{26}-Val-Glu-Ala	87
Human prourokinase	Pro-Pro-Glu-Glu_{143}~Leu_{144}-Lys-Phe-Gln	98
MMP-9		
Human type V collagen (α1)	Gly-Pro-Pro-Gly_{439}~Val_{440}-Val-Gly-Pro	99
Human type V collagen (α2)	Gly-Pro-Pro-Gly_{445}~Leu_{446}-Arg-Gly-Glu	99
Human type XI collagen (α1)	Gly-Pro-Gly-Gly_{439}~Val_{440}-Val-Gly-Pro	99
Human aggrecan	Ile-Pro-Glu-Asn_{341}~Phe_{342}-Phe-Gly-Val	89
Human galectin-3	Pro-Pro-Gly-Ala_{62}~Tyr_{63}-His-Gly-Ala	90
Human cartilage link	Arg-Ala-Ile-His_{16}~Ile_{17}-Gln-Ala-Glu	87
MMP-10		
Human cartilage link	Arg-Ala-Ile-His_{16}~Ile_{17}-Gln-Ala-Glu	87
Human cartilage link	Gly-Pro-His-Leu_{25}~Leu_{26}-Val-Glu-Ala	87

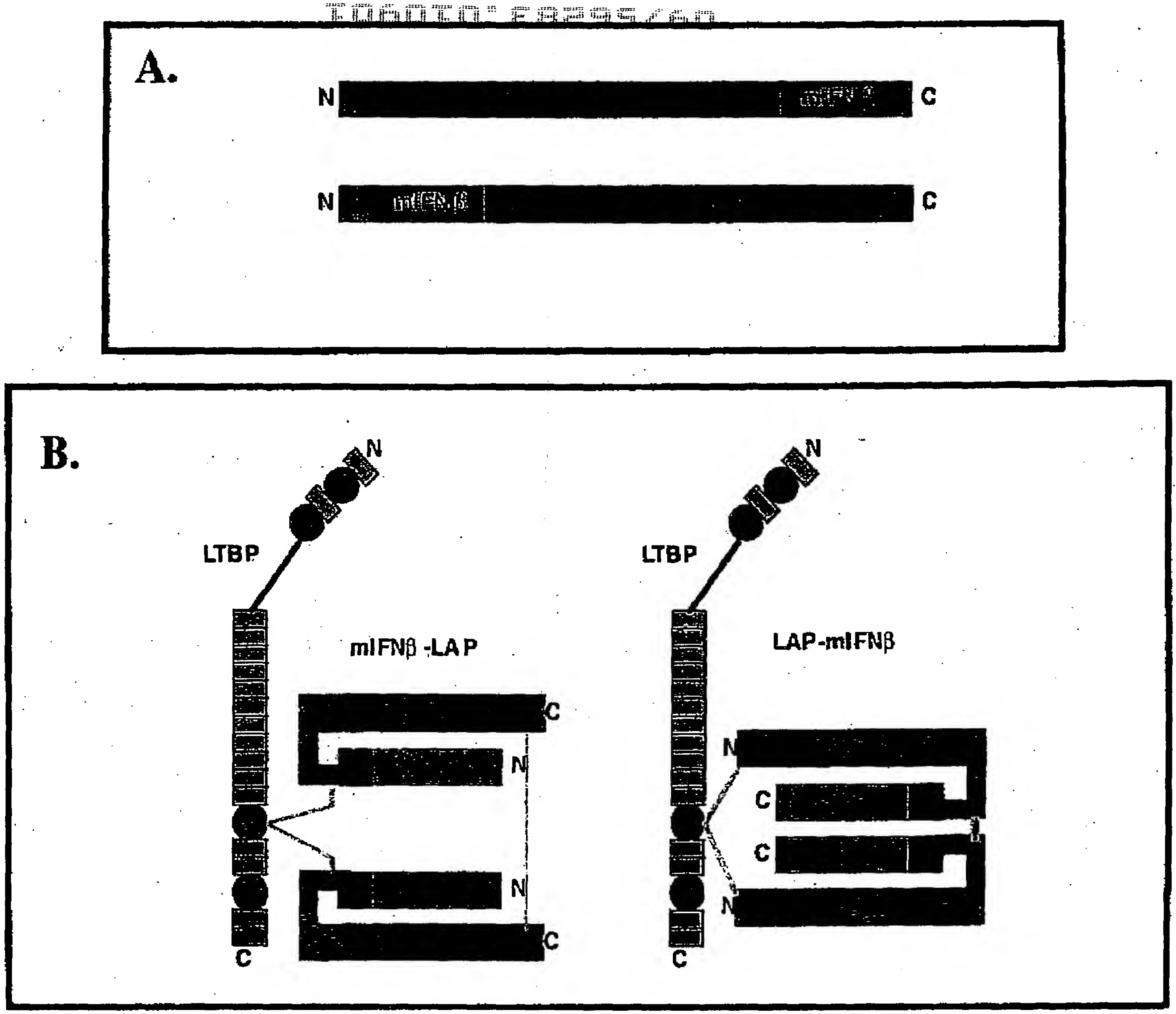

A.
N
mIFNβ
C
N
mIFNβ
C
B.
N
LTBP
mIFNβ -LAP
C
N
N
C
C
N
LTBP
LAP-mIFNβ
N
C
C
N
C

Fig. 5

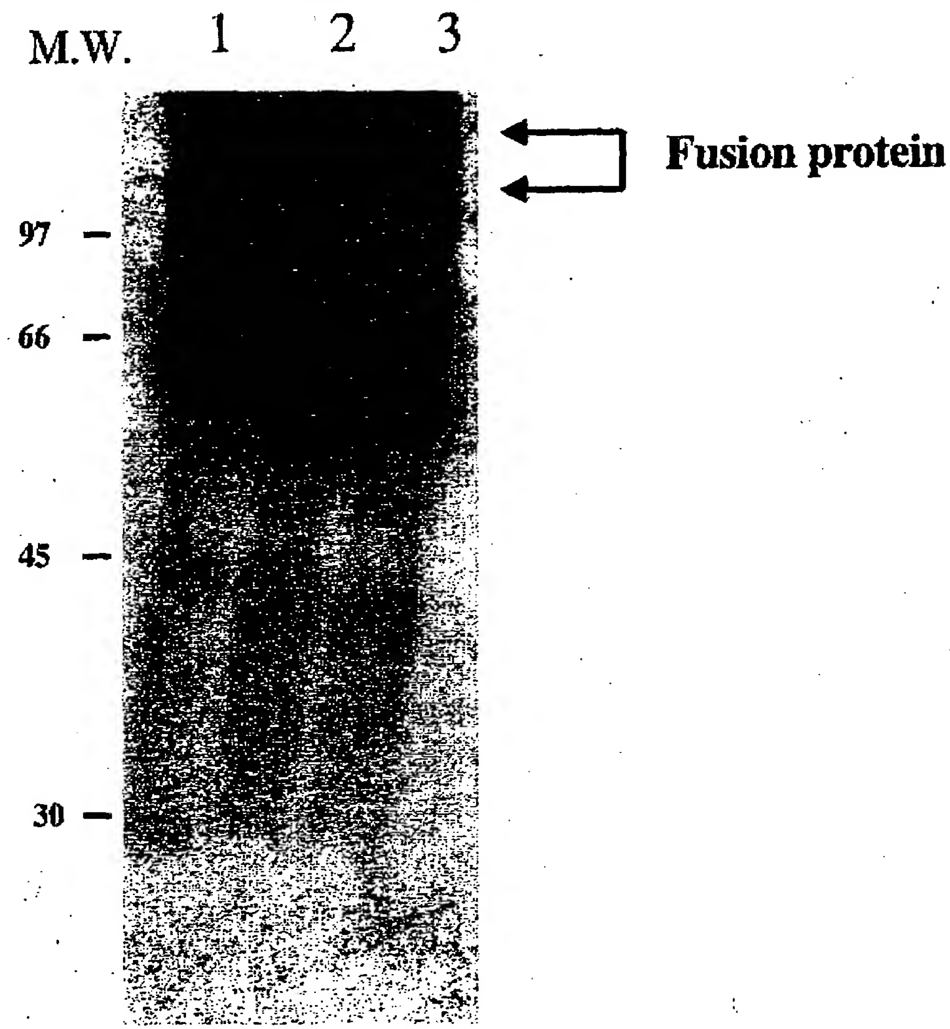
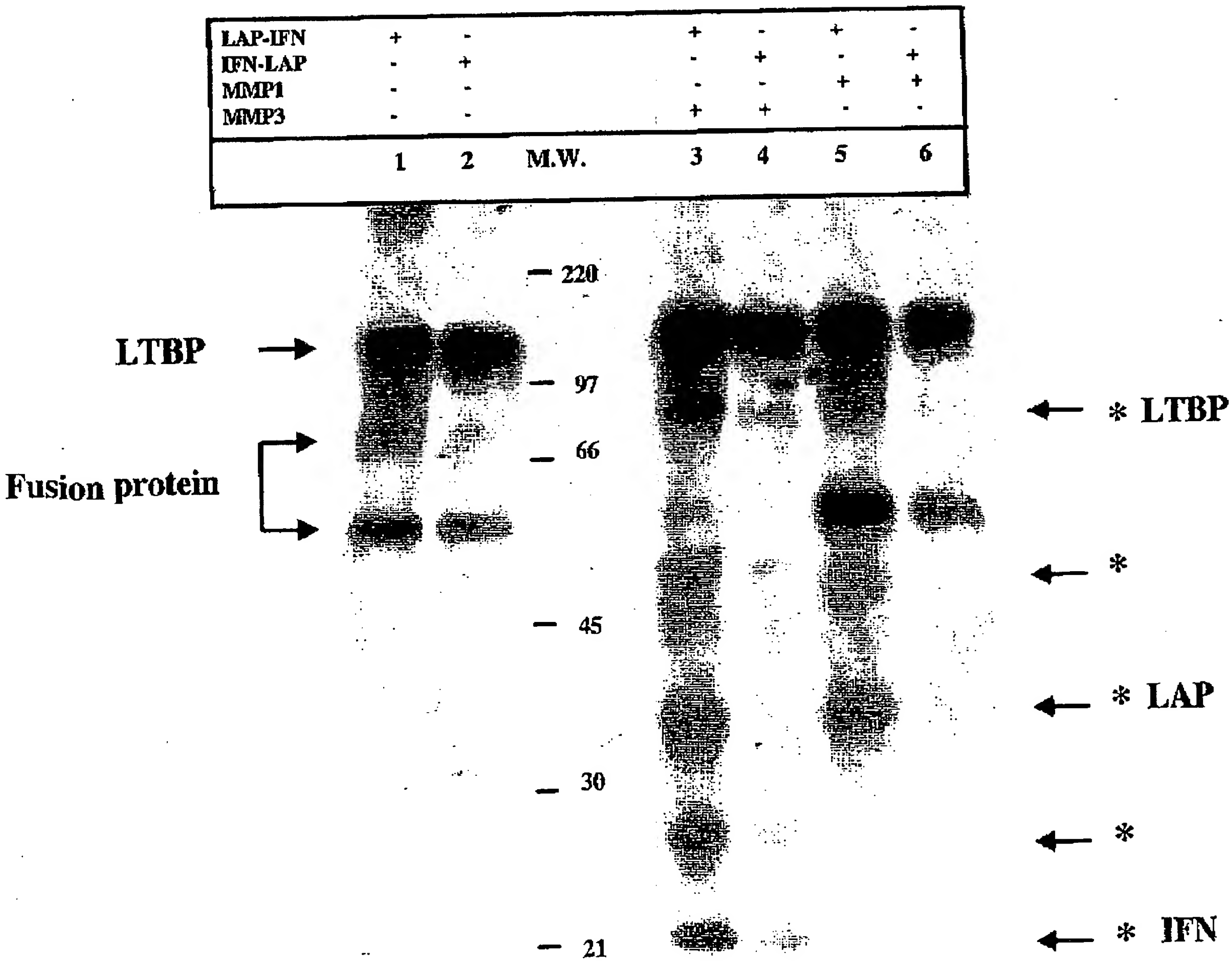
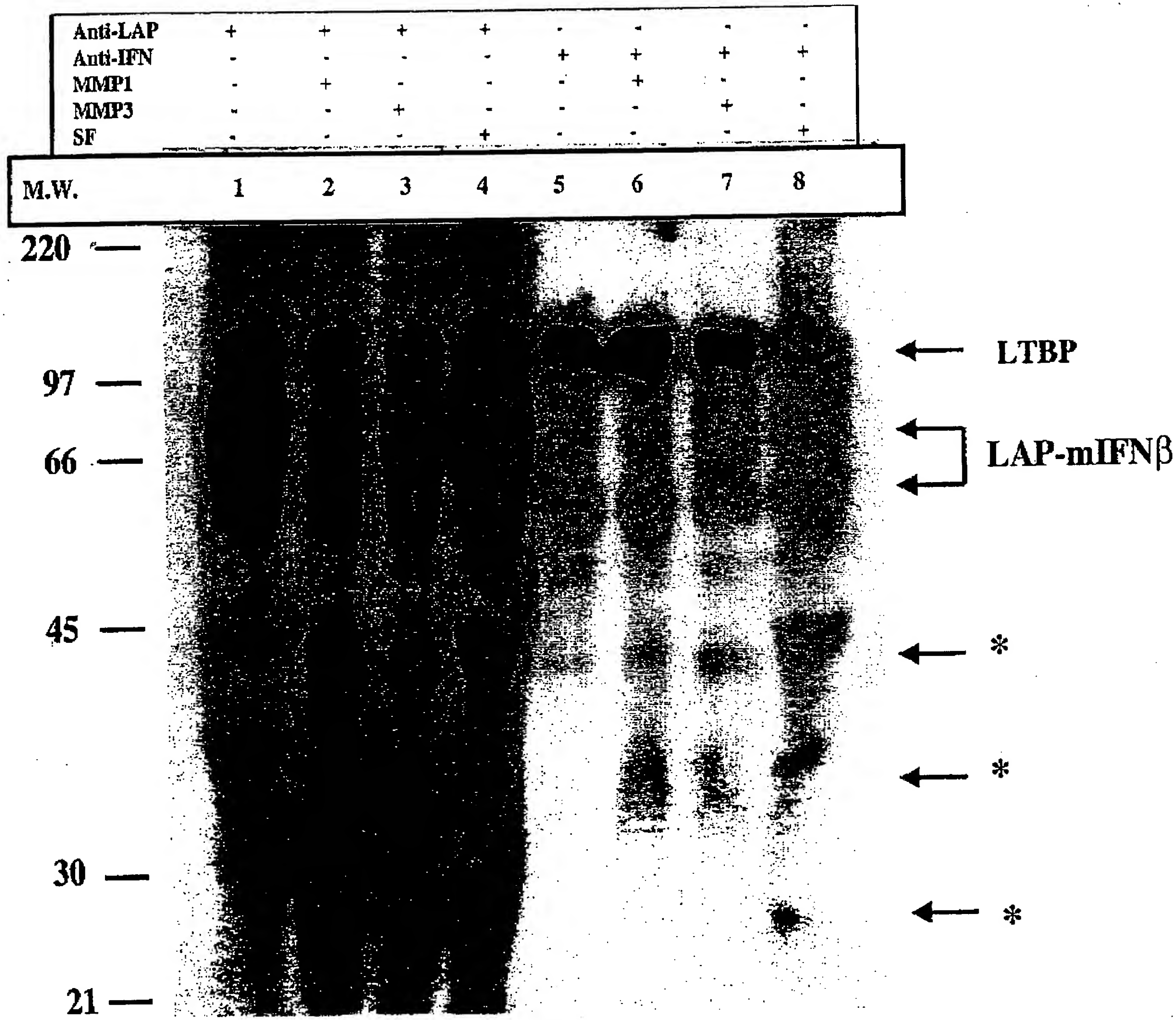


Fig. 6



LAP-IFN
IFN-LAP
MMP1
MMP3
1
2
M.W.
3
4
5
6
220
97
66
45
30
21
LTBP
Fusion protein
* LTBP
*
* LAP
*
* IFN

Fig. 7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Anti-IFN - - - - + + + +
MMP1 - + - - - + - -
MMP3 - - + - - - + -
SF - - - + - - - +
M.W. 1 2 3 4 5 6 7 8
220
97
66
45
30
21
LTBP
LAP-mIFNβ
*
*
*

Fig. 8a

Anti-LAP	+	+	+	+	-	-	-	-
Anti-IFN	-	-	-	-	+	+	+	+
MMP1	-	+	-	-	-	+	-	-
MMP3	-	-	+	-	-	-	+	-
SF	-	-	-	+	-	-	-	+
M.W.	1	2	3	4	5	6	7	8

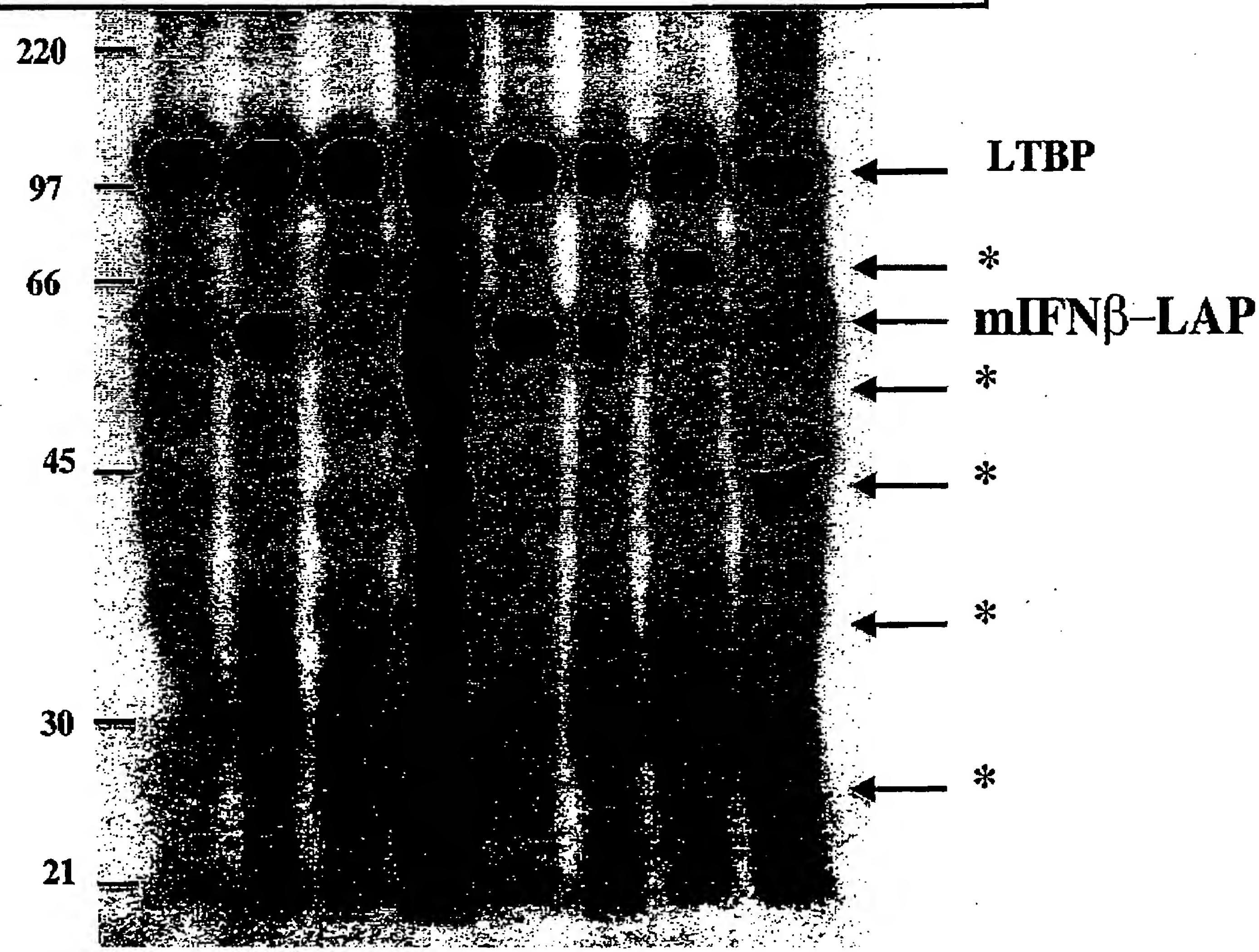
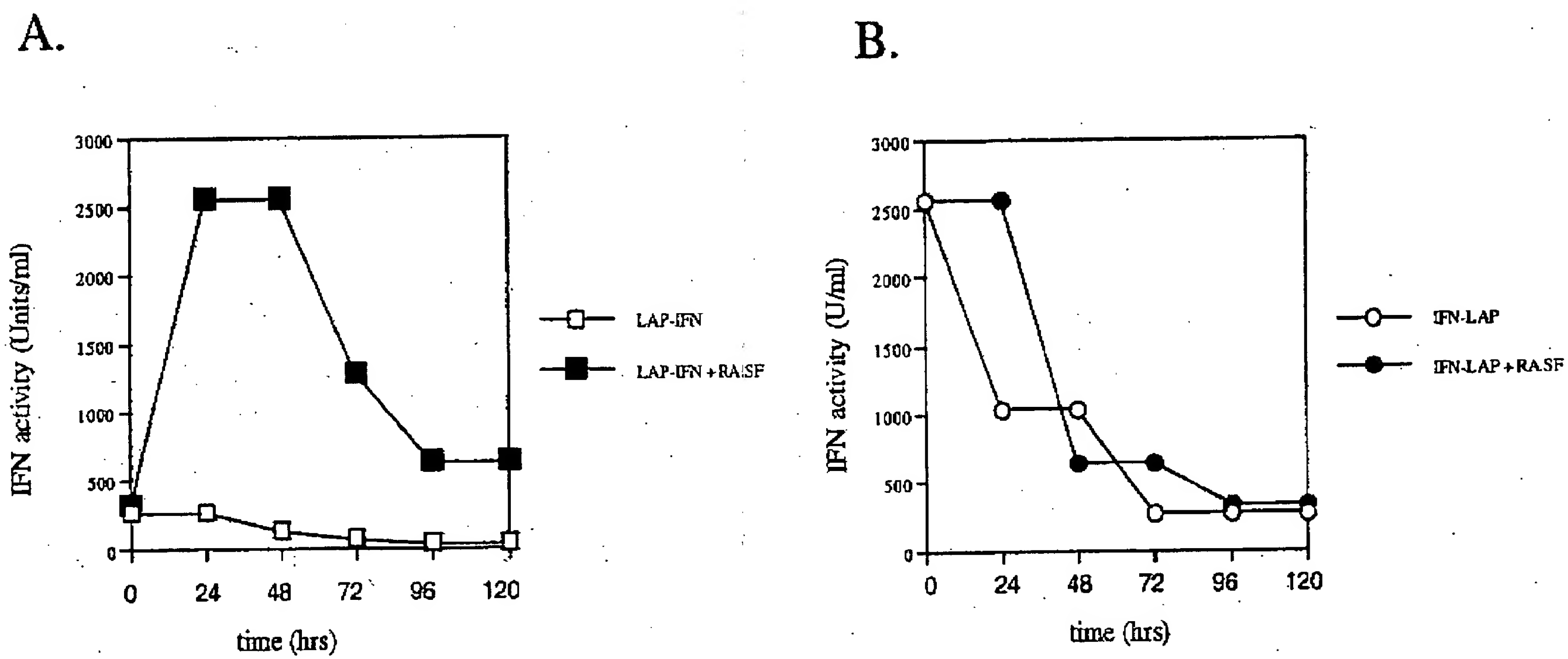


Fig. 8b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IFN activity (Units/ml)
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2500
2000
1500
1000
500
0
0
24
48
72
96
120
time (hrs)
LAP-IFN
LAP-IFN + RASF
B.
IFN activity (U/ml)
3000
2500
2000
1500
1000
500
0
0
24
48
72
96
120
time (hrs)
IFN-LAP
IFN-LAP + RASF

Fig. 9

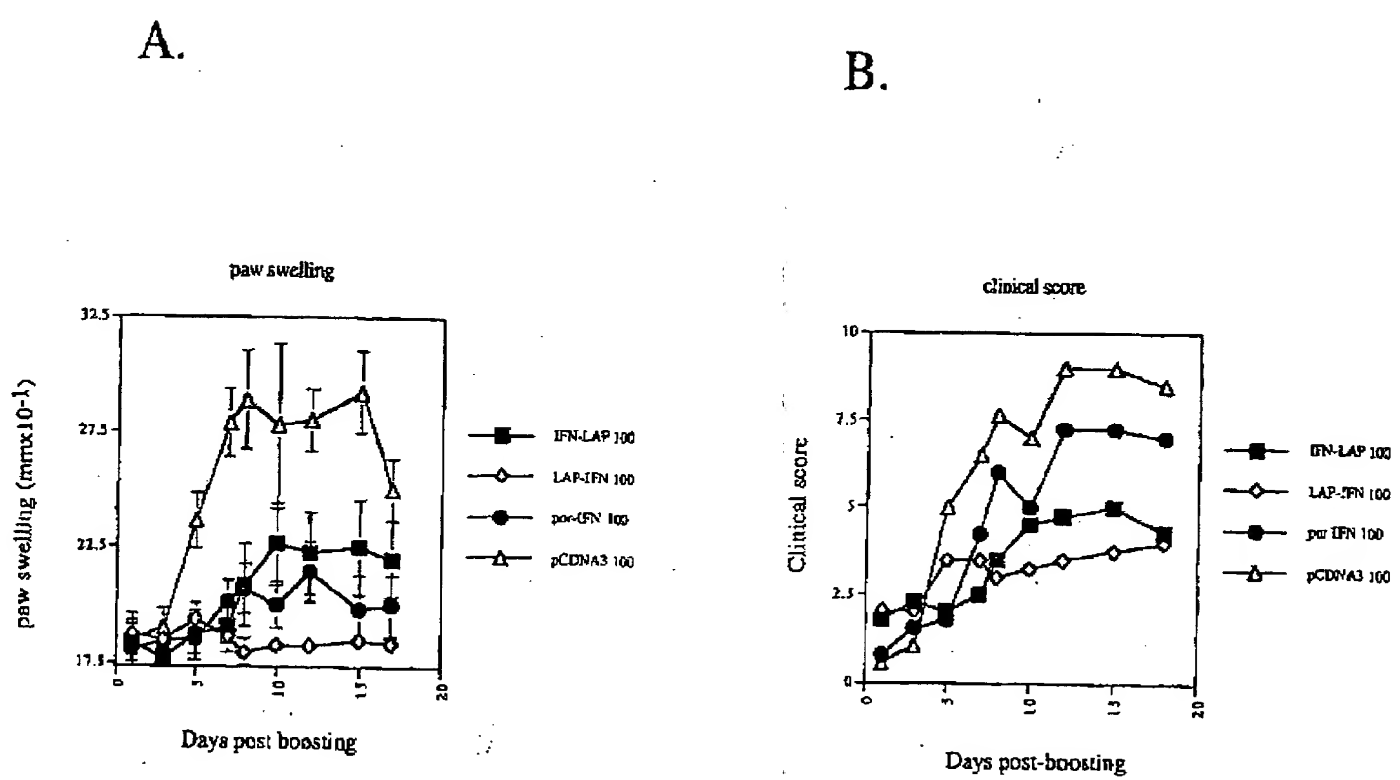
A.
paw swelling
paw swelling (mmx10-1)
32.5
27.5
22.5
17.5
0
5
10
15
20
Days post boosting
IFN-LAP 100
LAP-IFN 100
por-IFN 100
pCDNA3 100
B.
clinical score
Clinical score
10
7.5
5
2.5
0
0
5
10
15
20
Days post-boosting
IFN-LAP 100
LAP-IFN 100
por IFN 100
pCDNA3 100

Fig. 10